PROTEIN CRYSTAL ENGINEERING THROUGH DNA HYBRIDIZATION INTERACTIONS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the priority benefit under 35 U.S.C. § 119(e) of U.S. Provisional Patent Application No. 62/776,399, filed Dec. 6, 2018, which is incorporated herein by reference in their entirety.

STATEMENT OF GOVERNMENT INTEREST

[0002] This invention was made with government support under N00014-15-1-0043, awarded by the Office of Naval Research. The government has certain rights in the invention.

INCORPORATION BY REFERENCE OF MATERIAL SUBMITTED ELECTRONICALLY

[0003] The Sequence Listing, which is a part of the present disclosure, is submitted concurrently with the specification as a text file. The name of the text file containing the Sequence Listing is "2018-204_Seqlisting.txt", which was created on Dec. 6, 2019 and is 8,598 bytes in size. The subject matter of the Sequence Listing is incorporated herein in its entirety by reference.

FIELD OF THE INVENTION

[0004] The present disclosure provides compositions comprising protein crystals and methods for programmable biomaterial synthesis.

BACKGROUND

[0005] Chemists routinely design crystals with tunable topology, porosity, and reactive sites. However, structural biologists have not accomplished comparable feats with crystals comprised of biomacromolecules.[1-4] Protein crystals are a versatile class of materials for catalysis,[5] protein structure determination,[6] and separations,[7] however, they are often grown through trial-and-error approaches, as the complexity of protein-protein interactions (PPIs) limits their rational design.[8]

[0006] Protein crystals are an important class of biomaterials, however they are grown almost exclusively through trial-and-error methods and the final structure obtained is not designed, and cannot be controlled. Due to the complexity of protein-protein interactions (PPIs), no current method exists to design the structure of a single protein, or of multiple proteins, within protein crystals.

[0007] Through x-ray crystallography, protein single crystals enable fundamental understanding of protein structure and recognition [McRee, D. E. (1999). Practical protein crystallography (Elsevier); Rohs, R., Jin, X., West, S. M., Joshi, R., Honig, B., and Mann, R. S. (2010). Origins of Specificity in Protein-DNA Recognition. Annu. Rev. Biochem. 79, 233-269; Chothia, C., and Janin, J. (1975). Principles of protein—protein recognition. Nature 256, 705-708], and consequently have been important in the rational design of drugs [Mandal, S., Moudgil, M.n., and Mandal, S. K. (2009). Rational drug design. Eur. J. Pharmacol. 625, 90-100]. In addition, they have been used in chiral catalysis [Lalonde, J. J., Govardhan, C., Khalaf, N., Martinez, A. G., Visuri, K., and Margolin, A. L. (1995). Cross-linked crystals of Candida rugosa lipase: highly efficient catalysts for the resolution of chiral esters. J. Am. Chem. Soc. 117, 6845-6852] and enantiomeric separations [Vuolanto, A., Kiviharju, K., Nevanen, T. K., Leisola, M., and Jokela, J. (2003). Development of Cross-Linked Antibody Fab Fragment Crystals for Enantioselective Separation of a Drug Enantiomer. Cryst. Growth Des. 3, 777-782], and non-crystalline but ordered protein assemblies have been utilized to control cascade reactions [Fu, J., Yang, Y. R., Johnson-Buck, A., Liu, M., Liu, Y., Walter, N. G., Woodbury, N. W., and Yan, H. (2014). Multi-enzyme complexes on DNA scaffolds capable of substrate channelling with an artificial swinging arm. Nat. Nanotechnol. 9, 531; Wilner, O. I., Weizmann, Y., Gill, R., Lioubashevski, O., Freeman, R., and Willner, I. (2009). Enzyme cascades activated on topologically programmed DNA scaffolds. Nat. Nanotechnol. 4, 249-254; Niemeyer, C. M., Koehler, J., and Wuerdemann, C. (2002). DNA-Directed Assembly of Bienzymic Complexes from In Vivo Biotinylated NAD(P)H:FMN Oxidoreductase and Luciferase. ChemBioChem 3, 242-245]. However, protein crystallization is challenging because proteins are complex, dynamic molecules comprised of thousands of atoms [McPherson, A., and Gavira, J. A. (2013). Introduction to protein crystallization. Acta Crystallogr., Sect. F: Struct. Biol. Commun. 70, 2-20]. Furthermore, the interactions between protein surfaces that drive crystallization are weak, complex, and noncovalent, therefore, researchers interested in such structures have little control over crystallization and the type of crystals that form [Durbin, S. D., and Feher, G. (1996). Protein Crystallization. Annu. Rev. Phys. Chem. 47, 171-204].

[0008] Efforts to control protein crystallization have included modifications that affect charge [Cohen-Hadar, N., Lagziel-Simis, S., Wine, Y., Frolow, F., and Freeman, A. (2011). Re-structuring protein crystals porosity for biotemplating by chemical modification of lysine residues. Biotechnol. Bioeng. 108, 1-11; Simon, A. J., Zhou, Y., Ramasubramani, V., Glaser, J., Pothukuchy, A., Gollihar, J., Gerberich, J. C., Leggere, J. C., Morrow, B. R., Jung, C., et al. (2019). Supercharging enables organized assembly of synthetic biomolecules. Nat. Chem. 11, 204-212; Küunzle, M., Eckert, T., and Beck, T. (2016). Binary Protein Crystals for the Assembly of Inorganic Nanoparticle Superlattices. J. Am. Chem. Soc. 138, 12731-12734], hydrophobicity [Yamada, H., Tamada, T., Kosaka, M., Miyata, K., Fujiki, S., Tano, M., Moriya, M., Yamanishi, M., Honjo, E., Tada, H., et al. (2007). 'Crystal lattice engineering,' an approach to engineer protein crystal contacts by creating intermolecular symmetry: crystallization and structure determination of a mutant human RNase 1 with a hydrophobic interface of leucines. Protein Sci. 16, 1389-1397], protein structure [King, N. P., Bale, J. B., Sheffler, W., McNamara, D. E., Gonen, S., Gonen, T., Yeates, T. O., and Baker, D. (2014). Accurate design of co-assembling multi-component protein nanomaterials. Nature 510, 103-108; Brunette, T. J., Parmeggiani, F., Huang, P.-S., Bhabha, G., Ekiert, D. C., Tsutakawa, S. E., Hura, G. L., Tainer, J. A., and Baker, D. (2015). Exploring the repeat protein universe through computational protein design. Nature 528, 580-584; Doyle, L., Hallinan, J., Bolduc, J., Parmeggiani, F., Baker, D., Stoddard, B. L., and Bradley, P. (2015). Rational design of a-helical tandem repeat proteins with closed architectures. Nature 528, 585-588], ligand binding [Engilberge, S., Ren-